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Synthesis of novel azo compounds containing 5(4*H*)-oxazolone ring as potent tyrosinase inhibitors

Hooshang Hamidian [a,*], Roya Tagizadeh [a], Samieh Fozooni [b], Vahid Abbasalipour [a], Ali Taheri [c], Mohadeseh Namjou [d]

[a] Department of Chemistry, Payame Noor University (PNU), PO Box 19395-3697, Tehran, Iran
[b] Mining Engineering Department, Zarand High Education Center, Shahid Bahonar University, Kerman, Iran
[c] School of Advance Medical Science, Golestan University of Medical Science, Gorgan, Iran
[d] Department of Medical Laboratory, Golestan University of Medical Science, Gorgan, Iran

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ABSTRACT

Six new azo dyes containing of 5(4*H*)-oxazolone ring were prepared by diazotization of 4-aminohippuric acid and coupling with *N*,*N*-dimethylaniline, 1-naphthol and 2-naphthol and condensation with 4-fluoro benzaldehyde or 4-trifluoromethoxy benzaldehyde. The new compounds were fully characterized by spectroscopic techniques. All synthesized compounds exhibited high tyrosinase inhibitory behavior. The results of mushroom tyrosinase inhibition assays indicate that the 4-trifluoromethoxy derivatives have high degrees of inhibition and *N*,*N*-dimethylaniline derivatives are better for tyrosinase inhibition than 1-naphthol and 2-naphthol derivatives. All synthesized azo compounds (**4a–4f**) showed the most potent mushroom tyrosinase inhibition, comparable to that of Kojic acid and L-mimosine, as reference standard inhibitors.

1. Introduction

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1, syn. polyphenol oxidase), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants.[1] Tyrosinase inhibitors are clinically useful for the treatment of skin diseases associated with melanin hyperpigmentation and applied in cosmetics for whitening and depigmentation after sunburn.[2] Melanin is a heteropolymer of indole compounds and is produced inside melanosomes by the action of the tyrosinase enzyme on the tyrosinase precursor material in melanocytes. It has recently been discovered that some other factors such as metal ions and the TRP-1 and TRP-2 enzymes also contribute to the production of melanin. However, tyrosinase plays a critical role in the regulation of melanin biosynthesis. Therefore, many tyrosinase inhibitors that suppress melanogenesis have been widely studied with the aim of developing preparations for the treatment of hyperpigmentation.[3–7]

Nitrogen heterocycles are of special interest because they constitute an important class of natural and nonnatural products, many of which exhibit useful biological activities. Oxazolone derivatives are in general well-known five-membered nitrogen-containing heterocyclic compounds. 4-Arylidene-2-phenyl-5(4)-oxazolones are important intermediates for the synthesis of fine chemicals and precursors of several biologically active molecules such as amino acids and peptides.[8]

On the other hand, oxazolone derivatives are highly versatile intermediates used for the synthesis of several organic molecules, including amino acids, peptides, antimicrobial or antitumor compounds, immunomodulators, heterocyclic precursors for biosensors coupling, and photosensitive composition devices for proteins.[9–11]

It is well known that azo compounds are the widest class of industrial synthesized organic dyes due to their versatile application in various fields, such as dyeing textile fiber, biological–pharmacological activities and advanced application in organic synthesis.[12–16]

In recent years, the fabrication of azo dyes has been intensively investigated, due to their unique industrial applications in hypnotic drugs,[17] in living cells, in detecting cancer[18] and having pharmacological and biological activities.[19–22]

In this work, we synthesized a number of new azo compounds containing of oxazolone ring and studied chemical structures. Also we evaluated inhibitory effect on tyrosinase of new compounds.

2. Results and discussion

2.1. Chemistry

Diazonium salts could react readily with nucleophiles as an aromatic compounds containing amino or hydroxyl group, which have

* Corresponding author. Tel./fax: +98 341 3342465.
E-mail address: h_hamidian@pnu.ac.ir (H. Hamidian).

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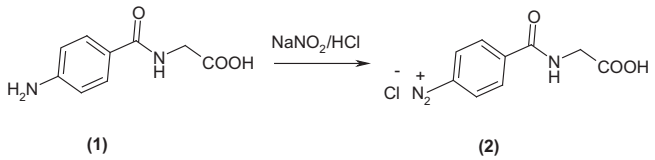


Scheme 1. Diazotisation of 4-aminohippuric acid.

been extensively researched and widely used for the preparation of molecules with significance for both academic and industrial applications. 4-Amino hippuric acid is dissolved in a 2.5% sodium carbonate solution by heating and stirring. In the solution of 4-amino hippuric acid, sodium nitrite is dissolved and 4-amino hippuric acid was diazotized by slow addition of concd HCl at 0 °C. A yellow precipitate of the diazonium salt (**2**) was formed (Scheme 1).[23]

Coupling components (*N*,*N*-dimethylaniline, 1-naphthol and 2-naphthol) are added to diazonium salt of 4-aminohippuric acid. Azo dyes (**3a**–**3c**) are produced in good yields. Diazonium salt is coupled to the *para*-position of the amine group, 2-position of hydroxyl group in 1-naphthol and 1-position of hydroxyl group in 2-naphthol (Scheme 2).

Then 4-arylidene-5(4*H*)-oxazolone azo dyes (**4a**–**4f**) are synthesized by classical Erlenmeyer reaction, involving condensation of compounds (**3a**–**3c**) with 4-fluorobenzaldehyde and 4-triflouromethoxy benzaldehyde in presence of acetic anhydride and sodium acetate under refluxing condition at 100 °C for 3 h (Scheme 3).

Generally, variation in color of these dyes results from the alternation in coupling components. Since the synthesized dyes obtained varied in color from red to brown, a convenient method of measuring the color of the compound was to study the absorption spectra of their solutions. The visible absorption maxima for the synthesized dyes were measured in Me_2SO at the concentration of 10^{-5} M and are listed in Table 1.

The absorption maxima of the synthesized dyes changed from 496 to 542 nm. Compounds (**4a**–**4f**) are stable solids whose structures were established by IR, ^{1}H NMR spectroscopy, mass spectrometry and elemental analysis.

2.2. Inhibitory activity of tyrosinase

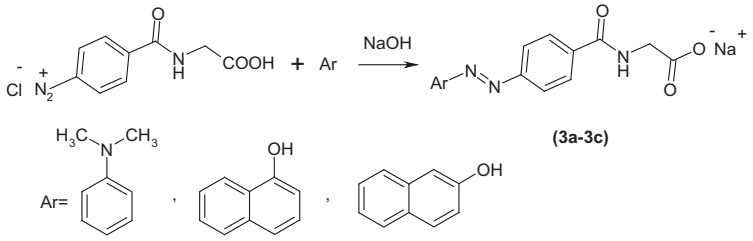
The compounds (**4a**–**4f**) demonstrated excellent in vitro tyrosinase inhibitory properties having IC_{50} values in the range of 4.33 ± 0.52 to 1.44 ± 0.36 μM, whereas standard inhibitors, L-mimosine and kojic acid, have IC_{50} values 3.68 ± 0.02 and 16.67 ± 0.52 μM, respectively (Table 2 and Fig. 1).

Compounds (**4a**) and (**4b**) having IC_{50} values 2.01 ± 0.39 and 1.44 ± 0.36, respectively, were found to be very active members of the series, even better than both the standard inhibitors. However, compounds (**4c**), (**4d**) and (**4e**) were found better than the standard kojic acid but not L-mimosine. 2-(4-{2-[4-(Dimethylamino)phenyl]-1-diazenyl}phenyl)-4-{1-[4-(trifluoromethoxy) phenyl]methylidene]-1,3-oxazol-5-one (**4b**) was found to be the most active one having IC_{50} = 1.44 ± 0.36 μM among all tested compounds.

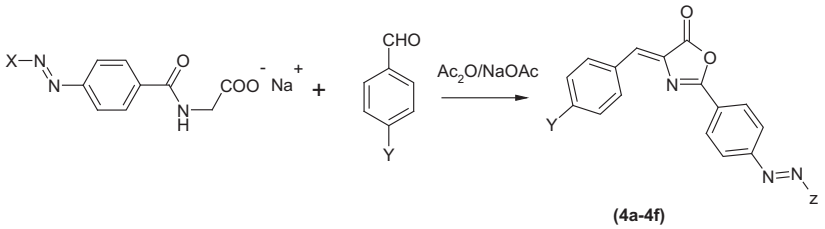
Comparing the activities with the structures of compounds, it turns out that the tyrosinase activity is mainly dependent on the substituents present at C-2 and C-4 positions of oxazolone ring. When tyrosinase inhibitory activity of the most active compound (**4b**) was compared with other compounds, it was observed that it has a 4-(trifluoromethoxy) phenyl group on the aliphatic double bond at C-4 and [4-(dimethylamino)phenyl]-1-diazenylphenyl group at C-2.

This shows that extension of conjugation through an aliphatic double bond could be the prerequisite for activity rather than extension through an aromatic ring.

A decrease in the activity of compounds (**4a**), (**4c**) and (**4e**) as compared to compounds (**4b**), (**4d**) and (**4f**) was due to the change in the substituent in phenyl ring present at C-4 of oxazolone ring. The least activity of compound (**4c**) (IC_{50} 4.33 ± 0.52 μM) may be

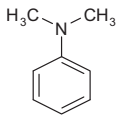
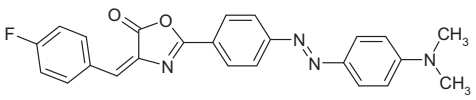
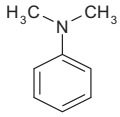
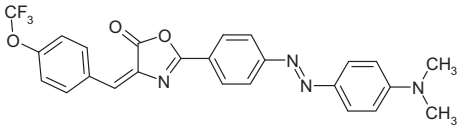
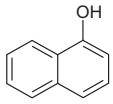
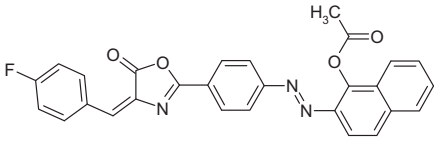
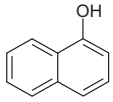
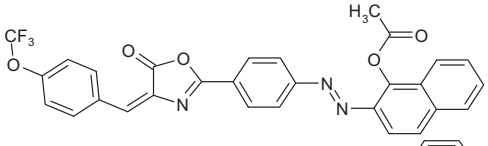
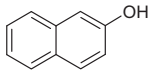
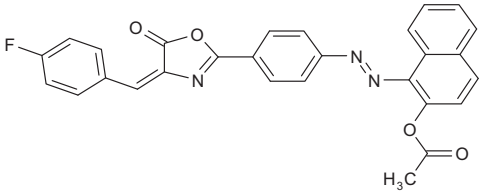
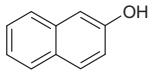
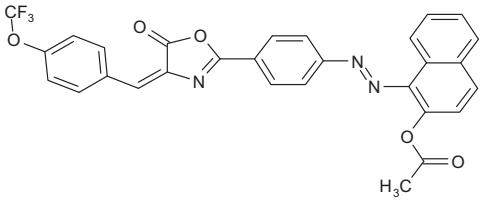


Scheme 2. Coupling of diazonium salt with aromatic compounds.



Scheme 3. Synthesis of fluoro 5(4*H*)-oxazolone azo dyes (**4a**–**4f**).

Table 1
Structure, yields and λ_{max} of new 5(4*H*)-oxazolone azo dyes (**4a–4f**)

Entry	R	Product		λ_{max} (Me_2SO)	Yield[a] (%)
1			**4a**	501	42
2			**4b**	542	47
3			**4c**	534	56
4			**4d**	517	61
5			**4e**	515	73
6			**4f**	510	69

[a] Isolated yields.

Table 2
Tyrosinase inhibitory activities of the compounds (**4a–4f**), as compared to the standard inhibitors

Entry	Compound	IC_{50} ± SEM[a] (μM)
1	**4a**	2.01 ± 0.39
2	**4b**	1.44 ± 0.36
3	**4c**	4.33 ± 0.52
4	**4d**	3.98 ± 0.16
5	**4e**	3.91 ± 0.24
6	**4f**	3.04 ± 0.31
7	Kojic acid[b]	16.67 ± 0.52
8	L-Mimosine[b]	3.68 ± 0.02

[a] SEM is the standard error of the mean.
[b] Standard inhibitors of the enzyme tyrosinase.

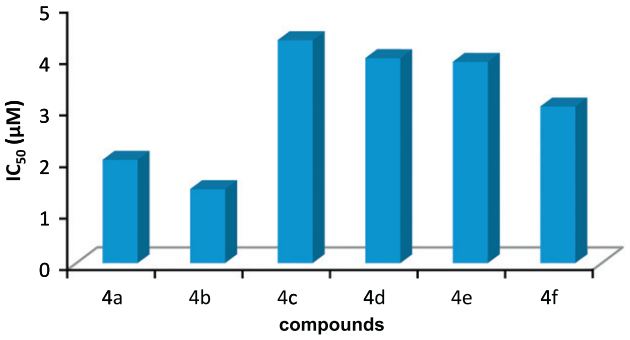


Figure 1. Comparative graphical presentation of the tyrosinase inhibitory potentials of the compounds (**4a–af**).

due to changing the substituent in phenyl rings present at C-4 and aromatic ring at C-2. Compound (**4b**) (IC_{50} 1.44 ± 0.36 μM) was found to be highly active member of the present series of azo compounds. Its excellent activity may be due to the presence of dimethylamino group in phenyl ring at C-2 and the presence of a trifluoromethoxy group in phenyl ring at C-4, which meets the criteria for achieving extension of conjugation. Compound (**4a**) (IC_{50} 2.01 ± 0.39 μM) is structurally similar to compound (**4b**) except where trifluoromethoxy group is replaced by fluoro. Interestingly, compounds (**4c–4f**) having IC_{50} values 4.33 ± 0.52, 3.98 ± 0.16, 3.91 ± 0.24 and 3.04 ± 0.31 μM, respectively, showed good activity. The activities may be due to the presence of electron-withdrawing substituent on phenyl ring at C-4 and electron-donating substituent on aromatic ring at C-2.

Effect of the compound **4b** on tyrosinase tertiary structure was considered by measurements of intrinsic fluorescence. We found that **4b** had a quenching effect on the intrinsic fluorescence, which gradually occurred with increasing concentration.

Table 3
Melanin production and cytotoxicity

Entry	Compound	Melanin production inhibition (%)	Cytotoxicity cell viability (%)
1	**4a**	35.70 ± 4.09	71.90 ± 2.87
2	**4b**	34.33 ± 3.21	67.26 ± 3.22
3	**4c**	31.31 ± 1.87	72.13 ± 1.33
4	**4d**	33.42 ± 1.48	73.52 ± 2.44
5	**4e**	32.50 ± 1.39	66.66 ± 5.31
6	**4f**	35.63 ± 0.74	68.71 ± 2.73
7	Kojic acid[a]	17.20 ± 1.22[b]	81.04 ± 1.23[b]

[a] Standard inhibitors of the enzyme tyrosinase.
[b] Tested at 200 μg/ml.

2.3. Melanin production inhibition and cytotoxicity

The inhibitory of the compounds (**4a**–**4f**) were also tested on melanin production and their cytotoxicity on B16F10 mouse melanoma cells at concentrations of 20 μg/ml. The results of melanin production inhibition and cytotoxicity by the compounds (**4a**–**4f**) are showed in Table 3. Compounds **4a**–**4f** prevented melanin production by 35.7%, 34.3%, 31.3%, 33.4%, 32.5% and 35.6%, respectively, at concentrations of 20 μg/ml. On the other hand, compounds (**4a**–**4f**) have shown moderate inhibition of melanin production. Cytotoxicity of new compounds (**4a**–**4f**) was evaluated and was defined that all compounds were relatively less toxic (Table 3).

3. Experimental

3.1. General

All the chemicals were obtained from Merck, Fluka, Sigma and Aldrich Companies and used without further purification. Melting points were measured using Thermo Fisher Scientific. IR spectra were recorded Bruker tensor 27, FT-IR Spectrophotometer. All ^{1}H NMR spectra were recorded on a Bruker 400 MHz Spectrophotometer. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Ultraviolet–visible (UV–vis) absorption spectra were recorded on an Perkin–Elmer spectrophotometer at the wavelength of maximum absorption (λ_{max}) in a range of DMSO at same concentrations (1×10^{-6}M). The mass Spectra were run on a Shimadzu Qp 5050 Ex Spectrometer. The microanalyses for C, H and N were performed on Perkin–Elmer elemental analyzer.

The fluorescence emission spectra were determined with a RF-5301PC Shimadzu spectrofluorometer using a cuvette with a 1 cm path length. An excitation wave length of 280 nm was used for the tryptophan fluorescence measurements, and the emission wavelength ranged between 300 and 420 nm.

3.2. Preparetion of diazonium salt of 4-aminohippuric acid (2)

In a 125-mL Erlenmeyer flask, 4-aminohippuric acid (0.01 mol) was added to 2% sodium carbonate solution (30 mL) until it was dissolved by boiling. The solution was then cooled and sodium nitrite (0.01 mol) was added, with stirring, until it was dissolved. The solution was cooled by placing it in an ice bath, and then concentrated hydrochloric acid (2 mL) and water (3 mL) were added. By acidifying the solution, a powdery yellow precipitate of the diazonium salt was separated.[23]

3.3. Sodium 2-[4-{2-[4-(dimethylamino)phenyl]-1-diazenyl} benzoyl amino] acetate (3a)

N,*N*-Dimethylaniline (0.01 mol) and glacial acetic acid (0.01 mol) was mixed. The solution of *N*,*N*-dimethylaniline acetate was added to suspension of diazotized hippuric acid, with stirring, and acid-stable form of the dye was separated. A stiff paste was formed in 5–10 min and then sodium hydroxide (5 g) was added to produce the orange sodium salt. The product was collected using saturated sodium chloride solution. The crude product was crystallized from water. Orange powder, decomposed >270 yield is 81%. IR (KBr): υ = 3354, 1716 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 3.07 (s, 6H, $2CH_3$), 3.61 (d, 2H, *J* 4.4 Hz, CH_2), 6.84 (d, 2H, *J* 8.9 Hz, ArH), 7.80–7.82 (m, 4H, ArH), 7.92 (br, 1H, NH), 7.98 (d, 2H, *J* 8.5, ArH) ppm. $C_{17}H_{17}O_3N_4Na$ (348) Calcd C 58.65, H 4.88, N 16.08. Found: C 58.54, H 4.92, N 16.18.[19]

3.4. Sodium 2-({4-[2-(1-hydroxy-2-naphthyl)-1-diazenyl]benzoyl}amino) acetate (3b)

1-Naphthol (0.01 mol) was dissolved in 5% sodium hydroxide solution (30 mL). The solution of 2-naphthol was added to suspension of diazotized hippuric acid, with stirring, and base-stable form of the dye was separated. A stiff paste was formed in 5–10 min and then 10 mL acetic acid 10% was added to produce the red sodium salt. The product was collected using saturated sodium chloride solution. The crude product was crystallized from water. The crude product was crystallized from water. Red powder, decomposed >236 yield is 81%. IR (KBr): υ = 3469, 3364, 1714 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 3.61 (d, 2H, *J* 4.4 Hz, CH_2), 6.88–8.63 (m, 12H, ArH, NH, OH) ppm. $C_{19}H_{14}N_3O_4Na$ (371) Calcd C 61.46, H 3.77, N 11.32. Found: C 61.73, H 3.66, N 11.09.

3.5. Sodium 2-({4-[2-(2-hydroxy-1-naphthyl)-1-diazenyl]benzoyl}amino) acetate (3c)

2-Naphthol (0.01 mol) was dissolved in 5% sodium hydroxide solution (30 mL). The solution of 2-naphthol was added to suspension of diazotized hippuric acid, with stirring, and base-stable form of the dye was separated. A stiff paste was formed in 5–10 min and then 10 mL acetic acid 10% was added to produce the red sodium salt. The product was collected using saturated sodium chloride solution. The crude product was crystallized from water. Red powder, decomposed >259 yield is 81%. IR (KBr): υ = 3477, 3355, 1710 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 3.64 (d, 2H, *J* 4.4 Hz, CH_2), 6.90–8.89 (m, 12H, ArH, NH, OH) ppm. $C_{19}H_{14}N_3O_4Na$ (371) Calcd C 61.46, H 3.77, N 11.32. Found: C 61.25, H 4.02, N 11.18.

3.6. General procedure for synthesis of compounds (4a–4f)

A mixture of anhydrous sodium acetate (0.01 mol), 4-fluoro benzaldehyde or 4-trifluoromethoxy benzaldehyde (0.01 mol), sodium salt of azo dye (**3a**–**3c**) (0.01 mol) and acetic anhydride (40 mL) was heated with stirring until the mixture is transformed from an orange semi-solid mass to a deep red liquid (2–4 h). After cooling, the precipitated product was filtered and recrystallized in toluene.

3.6.1. 2-(4-{2-[4-(Dimethylamino)phenyl]-1-diazenyl}phenyl)-4-[1-(4-fluorophenyl)methylidene]-1,3-oxazol-5-one (4a)

Dark red powder; mp: 264–266 °C. IR (KBr) *v*: 1789, 1657 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.15 (s, 6H), 6.78–8.30 (m, 13H). MS (EI) *m*/*z* (%): 42(100), 76(7), 104(2), 120(6), 171(4), 247(10), 275(6). Anal. Calcd for $C_{24}H_{19}N_4O_2F$: C, 69.57; H, 4.59; N, 13.53. Found: C, 69.66; H, 4.39; N, 13.71.

3.6.2. 2-(4-{2-[4-(Dimethylamino)phenyl]-1-diazenyl}phenyl)-4-{1-[4-(trifluoromethoxy)phenyl]methylidene]-1,3-oxazol-5-one (4b)

Brown powder; mp: 248–250 °C. IR (KBr) *v*: 1794, 1655 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.14 (s, 6H), 6.77–8.30 (m, 13H). MS (EI) *m*/*z* (%): 42(3), 76(17), 104(9), 119(100), 148(17), 224(3),

252(9), 480(M^+, 3). Anal. Calcd for $C_{25}H_{19}N_4O_3F_3$: C, 62.50; H, 3.96; N, 11.67. Found: C, 62.67; H, 4.11; N, 11.51.

3.6.3. 2-[2-(4-{4-[1-(4-Fluorophenyl)methylidene]-5-oxo-4,5-dihydro-1,3-oxazol-2-yl}phenyl)-1-diazenyl]-1-naphthyl acetate (4c)

Brown powder; mp: 246–248 °C. IR (KBr) *v*: 1800, 1726, 1654 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.53 (s, 3H), 7.19–9.04 (m, 15H). MS (EI) *m*/*z* (%): 42(100), 76(11), 91(9), 104(10), 143(51), 171(5), 247(3), 275(7). Anal. Calcd for $C_{28}H_{18}N_3O_4F$: C, 70.15; H, 3.76; N, 8.77. Found: C, 70.23; H, 3.59; N, 9.02.

3.6.4. 2-{2-[4-(5-Oxo-4-{1-[4-(trifluoromethoxy)phenyl]methylidene}-4,5-dihydro-1,3-oxazol-2-yl)phenyl]-1-diazenyl}-1-naphthyl acetate (4d)

Red powder; mp: 214–216 °C. IR (KBr) *v*: 1796, 1762, 1656 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.53 (s, 3H), 7.19–9.03 (m, 15H). MS (EI) *m*/*z* (%): 42(100), 91(75), 143(59), 171(11), 247(4), 275(17). Anal. Calcd for $C_{29}H_{18}N_3O_5F_3$: C, 63.85; H, 3.30; N, 12.07. Found: C, 63.92; H, 3.13; N, 12.33.

3.6.5. 1-[2-(4-{4-[1-(4-Fluorophenyl)methylidene]-5-oxo-4,5-dihydro-1,3-oxazol-2-yl}phenyl)-1-diazenyl]-2-naphthyl acetate (4e)

Dark red powder; mp: 280–282 °C. IR (KBr) *v*: 1790, 1763, 1652 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.37 (s, 3H), 7.19–8.76 (m, 15H). MS (EI) *m*/*z* (%): 42(100), 91(14), 104(4), 120(8), 143(44), 171(9), 274(7), 275(9). Anal. Calcd for $C_{28}H_{18}N_3O_4F$: C, 70.15; H, 3.76; N, 8.77. Found: C, 69.98; H, 3.96; N, 8.88.

3.6.6. 1-{2-[4-(5-Oxo-4-{1-[4-(trifluoromethoxy)phenyl]methylidene}-4,5-dihydro-1,3-oxazol-2-yl)phenyl]-1-diazenyl}-2-naphthyl acetate (4f)

Red powder; mp: 206–208 °C. IR (KBr) *v*: 1797, 1767, 1660 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.37 (s, 3H), 7.16–8.77 (m, 15H). MS (EI) *m*/*z* (%): 42(100), 104(4), 143(26), 171(4), 247(2), 275(6). Anal. Calcd for $C_{29}H_{18}N_3O_5F_3$: C, 63.85; H, 3.30; N, 12.07. Found: C, 64.06; H, 3.32; N, 11.91.

3.7. Biology

3.7.1. Tyrosinase inhibition assay

The spectrophotometric assay for tyrosinase was performed according to the method Ref. 20. Briefly, all the synthesized compounds were screened for the diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the compounds were dissolved in DMSO. The final concentration of DMSO in the test solution was 2.0%. Phosphate buffer, pH 6.8, was used to dilute the DMSO stock solution of test compounds. Thirty units of mushroom tyrosinase (0.5 mg/ml) were first pre-incubated with the compounds, in 50 mM phosphate buffer (pH 6.8), for 10 min at 25 °C. Then the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm of formation of the L-DOPA chrome for 10 min. The measurement was performed in triplicate for each concentration and averaged before further calculation. IC_{50} value, a concentration giving 50% inhibition of tyrosinase activity, was determined by interpolation of the dose–response curves. The percent of inhibition of tyrosinase reaction was calculated as following:

$$\text{Inhibition } (\%) = [B - S/B] \times 100$$

Here, the B and S are the absorbances for the blank and samples. All the experiments were carried out at least in triplicate and the results represent means ± SEM (standard error of the mean). Kojic acid and L-mimosine was used as reference standard inhibitors for comparison.

3.7.2. Inhibition of melanin production

Melanin production inhibition was ascertained by method of Wang et al.[24] A total of 8×10^4 cells were added to 60 mm plates, and were incubated at 37 °C in a CO_2 incubator then 10 μl test samples in DMSO were added to plates and were incubated for 72 h at 37 °C in a CO_2 incubator. After washing with PBS, cells were destroyed with 1 ml of 1 N NaOH, and 200 μl portions of raw cell extracts were moved to 96-well plates. Melanin production inhibition was determined by recording absorbance at 475 nm. The effects of test samples on melanin contents are stated as percent inhibitions of the value obtained in B16F10 mouse melanoma cells which were cultured with DMSO alone.

3.7.3. Cytotoxicity assay

Cytotoxicity assays were performed using a micro-culture MTT method described by Han et al.[25] A B16F10 mouse melanoma cell suspensionwas poured into a 96-well plate (10^3 cells/well) and cells were allowed to completely stick to each other overnight. Test samples were then added to the plate and were incubated at 37 °C for 72 h in a CO_2 incubator. 20 μl of MTT solution (2 mg/ml) was then added per well and incubated for 4 h. Supernatant was then removed and formazan was solubilized by adding 150 μl DMSO to each well with mild shaking. Absorbance at 490 nm was recorded using an ELISA plate reader.

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References and notes

1. Song, K. K.; Huang, H.; Han, P.; Zhang, C. L.; Shi, Y.; Chen, Q. X. *Biochem. Biophys. Res. Commun.* **2006**, *342*, 1147.
2. Lee, H. S. *J. Agric. Food Chem.* **2002**, *50*, 1400.
3. Masamoto, Y.; Ando, H.; Murata, Y.; Shimoishi, Y.; Tada, M.; Takahata, K. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 631.
4. Liu, J. B.; Yi, W.; Wan, Y. Q.; Ma, L.; Song, H. C. *Bioorg. Med. Chem.* **2008**, *14*, 1096.
5. Bandgar, P. B.; Adsul, L. K.; Chavan, H. V.; Shringare, S. N.; Korbad, B. L.; Jalde, S. S.; Lonikar, S. V.; Nile, S. H.; Shirfule, A. L. *Bioorg. Med. Chem.* **2012**, *20*, 5649.
6. Cho, J. C.; Rho, H. S.; Joo, Y. H.; Lee, C. S.; Lee, J.; Ahn, S. M.; Kim, J. E.; Shin, S. S.; Park, Y. H.; Suh, K. D.; Park, S. N. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4159.
7. Ghani, U.; Ullah, N. *Bioorg. Med. Chem.* **2010**, *18*, 4042.
8. Yu, C.; Zhou, B.; Su, W.; Xu, Z. *Synth. Commun.* **2006**, *36*, 3447.
9. Khan, K. M.; Mughal, U. R.; Khan, M. T. H.; Ullah, Z.; Perveen, S.; Choudhary, M. I. *Bioorg. Med. Chem.* **2006**, *14*, 6027.
10. Tozzini, V.; Bizzarri, A. R.; Pellegrini, V.; Nifosi, R.; Giannozzi, P.; Iuliano, A. *Chem. Phys.* **2003**, *287*, 33.
11. Ozturk, G.; Alp, S.; Timur, S. *Dyes Pigments* **2008**, *76*, 792.
12. Catino, S. C.; Farris, R. E. Azo dyes. In *Concise Encyclopedia of Chemical Technology*; Grayson, M., Ed.; John Wiley and Sons: New York, 1985; pp 142–144.
13. Fadda, A. A.; Etmen, H. A.; Amer, F. A.; Barghout, M.; Mohammed, Kh. S. *J. Chem. Technol. Biotechnol.* **1994**, *61*, 343.
14. Manuela, M.; Raposo, M.; Sousa Ana, M. R. C.; Mauricio, A.; Fonseca, C.; Kirsch, G. *Tetrahedron* **2005**, *61*, 8249.
15. Karci, F.; Demircali, A.; Sener, I. *Dyes Pigments* **2006**, *71*, 90.
16. Yazdanbakhsh, M. R.; Ghanadzadeh, A.; Moradi, E. *J. Mol. Liq.* **2007**, *136*, 165.
17. Cutting, W. C. *Handbook of Pharmacology*, 3rd ed.; Meredith Company: New York, 1967.
18. Izatt, R. M.; Christensen, J. H.; Rytting, J. H. *Chem. Rev.* **1972**, *72*, 439.
19. Zeng, H.; Lin, Z. P.; Sartorelli, A. C. *Biochem. Pharmacol.* **2004**, *68*, 911.
20. Sharma, P.; Rane, N.; Gurram, V. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4185.
21. Huang, C. Q.; Wilcoxen, K. M.; Grigoriadis, D. M.; McCarthy, J. R.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3943.
22. West, T. P. *Microbiol. Res.* **2004**, *159*, 29.
23. Fozooni, S.; Momeni, A.; Hamidian, H.; Khabazzadeh, H. *Arkivoc* **2008**, *xiv*, 115.
24. Wang, H. M.; Chen, C. Yi.; Chen, C. Y.; Ho, M. L.; Chou, Y. T.; Chang, H. C.; Lee, C. H.; Wang, C. Z.; Chu, I. M. *Bioorg. Med. Chem.* **2010**, *18*, 5241.
25. Han, J.; Ma, I.; Hendzel, M. J.; Turner, J. A. *Breast Cancer Res.* **2009**, *11*, R57.